

# LncRNA 00152 promotes the development of hepatocellular carcinoma by activating JAK2/STAT3 pathway

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**Abstract. – OBJECTIVE:** The aim of this study was to explore the regulatory role of lncRNA 00152 and JAK2/STAT3 pathway in the pathogenesis of hepatocellular carcinoma (HCC), and to investigate the possible underlying mechanism.

**PATIENTS AND METHODS:** Expression levels of lncRNA 00152 in HCC tissues, matched para-cancerous tissues and normal liver tissues were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), respectively. The correlation between lncRNA 00152 expression and pathological characteristics of HCC patients was analyzed. Meanwhile, the expression level of lncRNA 00152 in HCC cell lines was detected by qRT-PCR. After knockdown or overexpression of lncRNA 00152 in MHCC97 or HB611 cells, the proliferative ability and cell cycle were detected by EdU assay and flow cytometry, respectively. Also, Western blot was conducted to detect the protein expression levels of JAK2 and STAT3 in MHCC97 and HB611 cells.

**RESULTS:** The expression of lncRNA 00152 in HCC tissues was significantly higher than that of matched para-cancerous tissues and normal liver tissues. lncRNA 00152 expression was positively correlated with tumor stage and tumor size, whereas negatively correlated with the overall survival of HCC patients. High expression of lncRNA 00152 might be a potential hallmark for the diagnosis of HCC, with the AUC of 0.8425. Similarly, lncRNA 00152 was highly expressed in HCC cell lines when compared with that of normal liver cells. Knockdown of lncRNA 00152 in MHCC97 cells remarkably decreased the proliferative ability and arrested cell cycle. Overexpression of lncRNA 00152 in HB611 cells significantly promoted cell proliferation and cell cycle. Furthermore, Western blot re-

sults showed that lncRNA 00152 knockdown up-regulated the protein expression levels of JAK2 and STAT3 in HCC cells.

**CONCLUSIONS:** High expression of lncRNA 00152 promotes the development of HCC by activating the JAK2/STAT3 pathway.

*Key Words:*

lncRNA 00152, JAK2/STAT3 pathway, HCC, Proliferation.

## Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, with extremely high morbidity and mortality<sup>1</sup>. Several therapies have been developed for the treatment of HCC, including surgery, radiotherapy, chemotherapy, and molecular targeted therapy. However, the 5-year survival of HCC is relatively low due to a high degree of malignancy and rapid progression<sup>2,3</sup>. Therefore, it is of great significance to reveal the pathogenesis of HCC, eventually developing new therapeutic targets and improving clinical outcomes of affected patients.

lncRNA is a type of non-coding RNA with about 200-100,000 nt in length. It does not encode proteins, but can regulate various processes. It is known to all that lncRNA regulates gene expression at multiple levels<sup>4,5</sup>. A large number of biological functions can be regulated by lncRNAs, such as transcriptional activation, X-chromosome silencing, chromatin modification, and genom-

ic imprinting. Previous studies<sup>6,7</sup> have indicated the regulatory effect of lncRNAs on multiple diseases, including tumors, Huntington's disease and Alzheimer's disease. In recent years, many tumor-associated lncRNAs have been discovered. These lncRNAs may affect the growth, apoptosis, invasion, and metastasis of tumor cells, which can serve as therapeutic targets. For example, lncRNA-ROR induces the transition of epithelial cells to mesenchymal cells, thereafter promoting tumorigenesis and metastasis of breast cancer<sup>8</sup>. lncRNA-p21 enhances the radiosensitivity of human colorectal cancer through Wnt/ $\beta$ -catenin pathway<sup>9</sup>. Meanwhile, lncRNA-FEZ1-AS1 serves as an oncogene in gastric cancer, and its low expression may promote the proliferation of gastric cancer cells by inhibiting p21<sup>10</sup>. lncRNA has also been found to exert a crucial role in the occurrence and development of HCC<sup>11-13</sup>. For example, lncRNA-HEIH is highly expressed in liver cancer tissues, which is correlated with the recurrence rate and survival time<sup>14</sup>. Therefore, lncRNA can be used as an important indicator for comprehensive treatment of liver cancer.

The JAKS/STATs pathway, which is composed of the JAKS family and the STATs family, is important in regulating cytokines, chemokines, and growth factor receptors. JAKS (Janus kinase) is a type of receptor tyrosine kinase containing JAK1-3 and TYK2. STAT (signal transducer and activators of transcription) family consists of seven members, namely STAT1-4, STAT5a, STAT5b, and STAT6. STAT is catalyzed by JAKS and transferred to the nucleus, further regulating the transcription and translation of specific genes. Under normal conditions, the JAKS/STATs pathway is tightly regulated and transiently activated. Abnormal activation of the JAKS/STATs pathway is critical in tumor metastasis, proliferation, migration, invasion, and angiogenesis. Several studies<sup>15,16</sup> have shown that the JAKS/STATs pathway is closely associated with various tumor-related pathways, and is the convergence point of these pathways. STAT3, a key factor in the JAKS/STATs pathway, has been identified as an oncogene<sup>17</sup>. Meanwhile, STAT3 activation is associated with the upregulation of cyclin D1 and cMyc, which accelerates cell cycle progression and promotes cell proliferation<sup>18</sup>. Some studies have also indicated that p-STAT3 overexpression enhances the invasion and metastasis of cutaneous squamous cell carcinoma<sup>19</sup>. Furthermore, targeted inhibition of STAT3 can suppress cell invasion and metastasis *in vivo* and *in vitro*<sup>20</sup>.

In summary, lncRNA and the JAK2-STAT3 pathway are closely related to the pathogenesis of malignant tumors. Therefore, exploration of the relationship between lncRNA and the JAK2-STAT3 pathway may provide new directions for HCC treatment.

## Patients and Methods

### Sample Collection

From April 2016 to December 2017, 58 pairs of HCC and para-cancerous tissues were collected from HCC patients who underwent surgical resection in the Affiliated Yantai Yuhuangding Hospital of Qingdao University. Para-cancerous tissues were harvested 3 cm away from the tumor edge without tumor cell infiltration. Meanwhile, 22 normal liver tissues were harvested from organ donors. Collected tissues were immediately preserved in liquid nitrogen for subsequent experiments. Enrolled HCC patients did not receive preoperative treatments and were pathologically diagnosed after surgery. The informed consent was obtained from each subject before the study. This investigation was approved by the Ethics Committee of the Hospital.

### Cell Culture

HCC cell lines (MHCC97, Huh7, and HB611) and normal liver cell line (LO2) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). LO2 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). However, HCC cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% FBS. All cells were maintained in a 37°C, 5% CO<sub>2</sub> incubator. When the confluence was up to 80%, cell passage was performed.

### Cell Transfection

HCC cells were first seeded into 24-well plates at a density of  $1 \times 10^5$  cells per well. HB611 cells were transfected with pcDNA3.1-lncRNA 00152 or pcDNA3.1 vector. Meanwhile, MHCC97 cells were transfected with si-lncRNA 00152-1, si-lncRNA 00152-2, si-lncRNA 00152-3 or negative control, respectively. Cell transfection was performed in strict accordance with the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

### **5-Ethynyl-2'-Deoxyuridine (EdU) Proliferation Assay**

Transfected cells were incubated with 500  $\mu\text{mol/L}$  EdU (RiboBio, Nanjing, China) for 2 h. Subsequently, the cells were washed three times with PBS and fixed with PFA for 30 min. Cells were then decolorized with 2 g/L glycine for 5 min, and washed with phosphate-buffered saline (PBS) containing 0.5% Triton X-100 for 10 min. Next, the cells were stained with Apollo for 30 min in the dark, followed by Hoechst 33342 staining for another 30 min. Finally, EdU-positive cells were detected by a fluorescence microscope.

### **Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)**

Total RNA of cells was extracted according to the instructions of the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, extracted RNA was reversely transcribed into complementary deoxyribonucleic acid (cDNA). QRT-PCR was then performed to detect the expression levels of related genes. QRT-PCR reaction parameters were as follows: denaturalization at 95°C for 60 s, extension at 95°C for 30 s and annealing at 60°C for 40 s, for a total of 40 cycles. Relative gene expression was calculated by the  $2^{-\Delta\Delta C_t}$  method. Primers used in this study were as follows: GAPDH: forward: 5'-AAGGGTGAAG-GTCGGAGTCAA-3', reverse: 5'-AATGAAGG-GGTCATTGATGG-3'; LncRNA: 00152 forward: 5'-CACTGAAAATCACGACTCC-3', reverse: 5'-AAATGGGAAACCGACCAGAC-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'.

### **Western Blot**

Transfected cells were lysed with cell lysis buffer. The concentration of extracted protein was detected by the bicinchoninic acid (BCA) protein assay kit (Abcam, Cambridge, MA, USA). Subsequently, total proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk, the membranes were incubated with primary antibody (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. The membrane was incubated with the secondary antibody after rinsing with the buffer solution (TBST). Finally, immunoreactive bands were exposed by the enhanced chemiluminescence (ECL) method (Thermo Fisher Scientific, Waltham, MA, USA).

### **Cell Cycle**

HCC cells in good growth were digested for preparing a cell suspension. After centrifugation, the cells were re-suspended in 0.1 mL pre-cooled PBS and 1 mL pre-cooled 75% ethanol. Subsequently, the suspension was centrifuged at 4°C, 1000 rpm/5 min twice, followed by the incubation with PI and RNase A at a final dose of 50 ng/mL. After 30 minutes of water bath at 37°C in the dark, cell cycle was detected by flow cytometry.

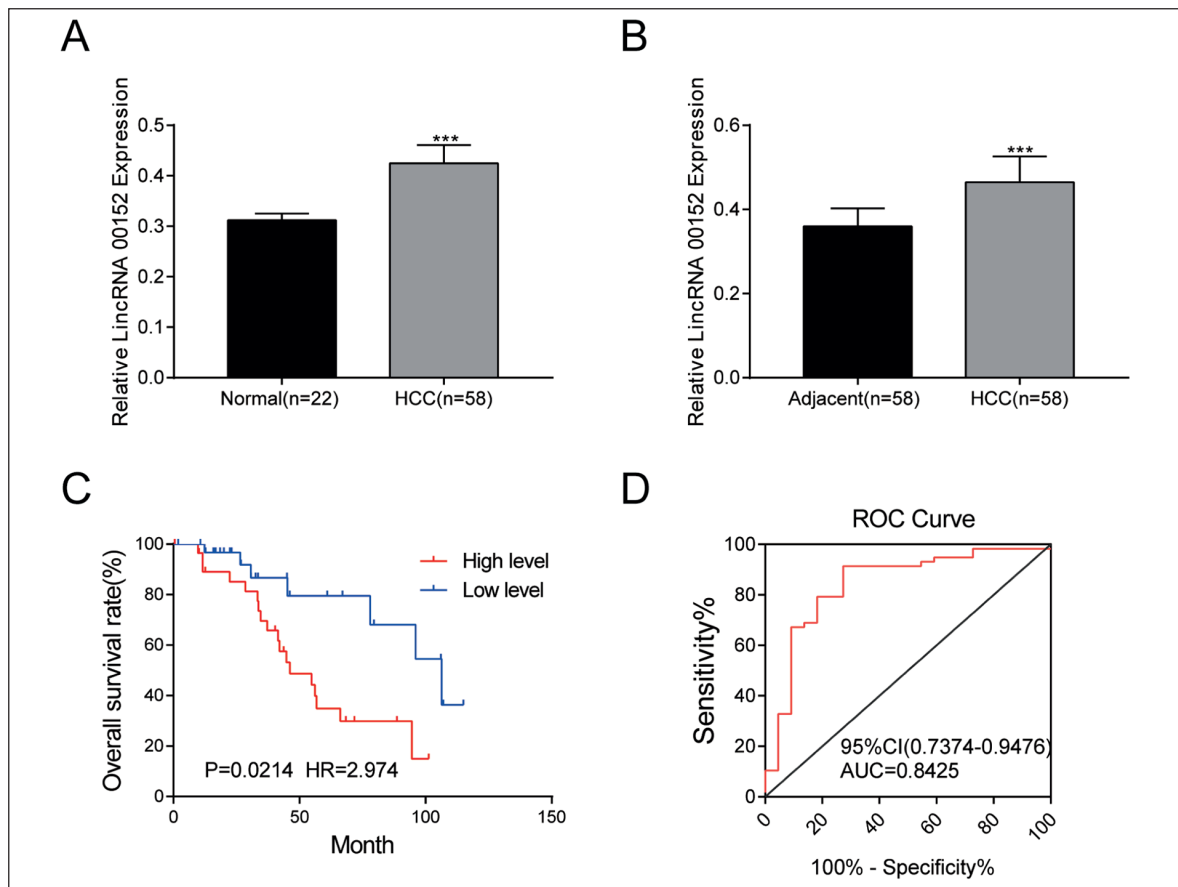
### **Statistical Analysis**

Statistical Product and Service Solutions (SPSS 19.0) Software (IBM, Armonk, NY, USA) was used for all statistical analysis. Quantitative data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). The *t*-test was used to compare the differences between the two groups. Categorical data were compared using the  $\chi^2$ -test.  $p < 0.05$  was considered statistically significant.

## **Results**

### **LncRNA 00152 Was Highly Expressed in HCC**

We first detected the expression level of lncRNA 00152 in HCC tissues, and then, we matched para-cancerous tissues and normal liver tissues by qRT-PCR. Results showed that the expression of lncRNA 00152 in 58 HCC tissues was significantly higher than that of normal liver tissues (Figure 1A). Similarly, lncRNA 00152 was highly expressed in HCC tissues than that of matched para-cancerous tissues (Figure 1B). The basic characteristics of enrolled HCC patients were collected, including age, sex, tumor size, TNM stage, tumor location, and lymph node metastasis (Table I). Correlation analysis demonstrated that lncRNA 00152 expression was positively correlated with TNM stage and tumor size in HCC patients. Meanwhile, HCC patients with higher expression of lncRNA 00152 presented evident shorter overall survival than those with lower expression (Figure 1C). Based on the different expression of lncRNA 00152 in HCC and para-cancerous tissue of each patient, ROC was conducted and AUC (area under curve) was calculated. Results demonstrated that the AUC was 0.8425 (95% CI: 0.7374-0.9476), indicating the diagnostic value of lncRNA 00152 in HCC (Figure 1D).



**Figure 1.** LncRNA 00152 was highly expressed in HCC. **A**, LncRNA 00152 was highly expressed in 58 HCC tissues than that of normal liver tissues. **B**, LncRNA 00152 was highly expressed in HCC tissues than that of matched para-cancerous tissues. **C**, LncRNA 00152 expression was positively correlated with TNM stage and tumor size, whereas negatively correlated with the overall survival of HCC patients. **D**, ROC of LncRNA 00152 expression in HCC tissues and para-cancerous tissues (AUC=0.8425, 95% CI: 0.7374-0.9476).

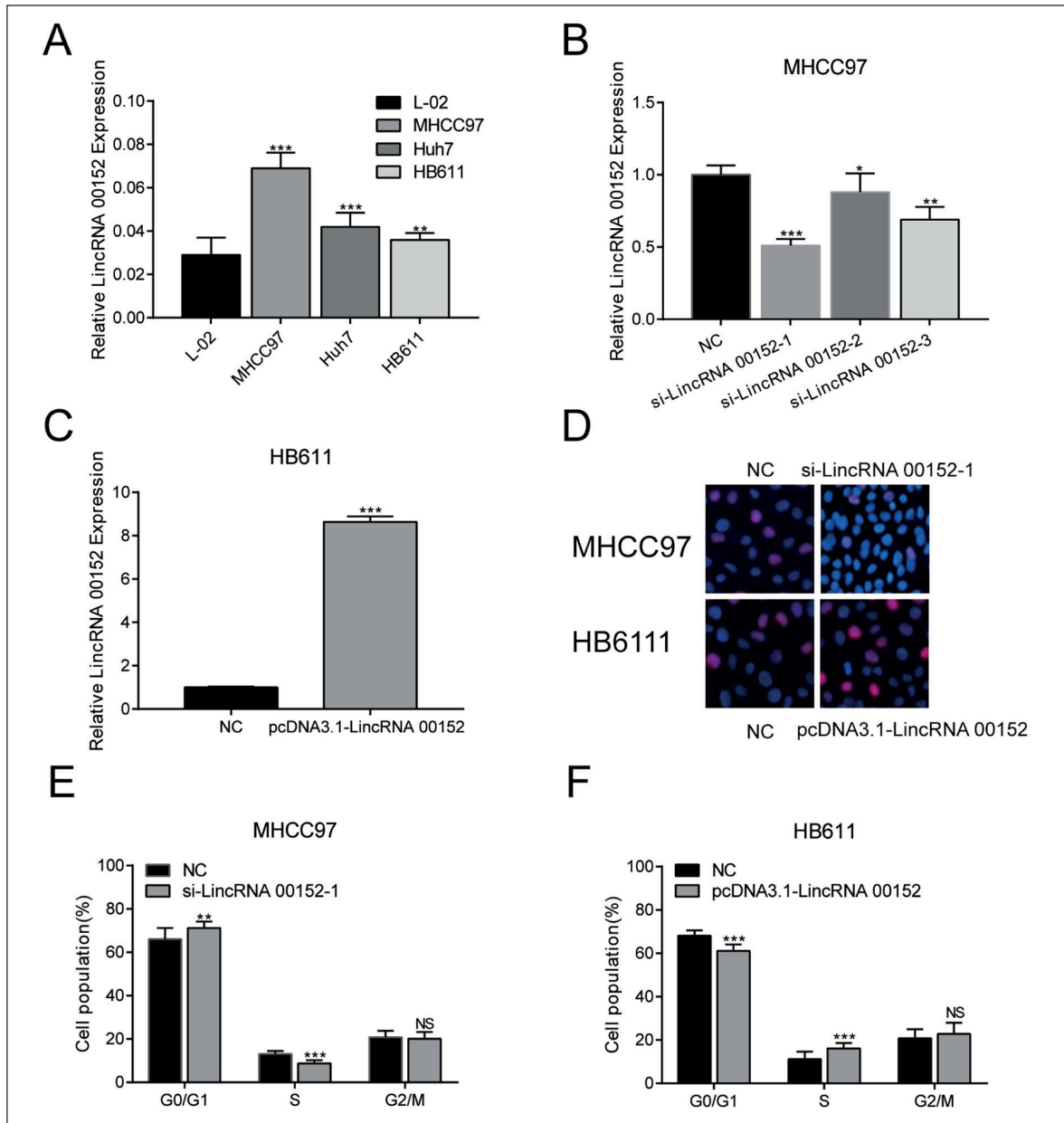
**Table I.** Basic characteristics of enrolled HCC patients (n = 58).

Clinicopathologic features	Number (n = 58)	LincRNA 00152 expression		p-value
		Low (n = 29)	High (n = 29)	
Age (years)				0.5806
≤ 60	20	11	9	
> 60	38	18	20	
Gender				0.7884
Male	23	12	11	
Female	35	17	18	
Tumor size				0.0083*
≤ 5 CM	26	18	8	
> 5 CM	32	11	21	
TNM stage				0.0157*
I-II	23	16	7	
III-IV	35	13	22	
Histological classification				0.7884
Low grade	23	12	11	
Medium and high grade	35	17	18	
Lymph node metastasis				0.4207
Absent	23	10	13	
Present	35	19	16	

### LncRNA 00152 Promoted the Proliferative Ability of HCC Cells

Subsequently, we detected the lncRNA 00152 expression in HCC cell lines (MHCC97, Huh7, and HB611) and normal liver cell line (LO2). QRT-PCR results found that lncRNA 00152 was

highly expressed in HCC cells than that of normal liver cells (Figure 2A). Among the three HCC cell lines, MHCC97 cells expressed the highest level and HB611 cells expressed the lowest level of lncRNA 00152. Then, three lines of si-lncRNA 00152 were transfected into MHCC97



**Figure 2.** LncRNA 00152 promoted the proliferative ability of HCC cells. **A**, LncRNA 00152 was highly expressed in HCC cells than that of normal liver cells. **B**, Transfection efficiencies of three lines of si-lncRNA 00152 in MHCC97 cells. **C**, Higher expression of lncRNA 00152 was found in HB611 cells transfected with pcDNA3.1-lncRNA 00152 than those transfected with pcDNA3.1 vector. **D**, EdU assay suggested that lncRNA 00152 overexpression remarkably stimulated the proliferative ability of HB611 cells. LncRNA 00152 knockdown remarkably inhibited the proliferative ability of MHCC97 cells. **E**, Knockdown of lncRNA 00152 in MHCC97 cells remarkably increased the number of cells in the G0/G1 phase but decreased the number of cells in S phase, demonstrating cell cycle arrest. **F**, Overexpression of lncRNA 00152 in HB611 cells significantly decreased the number of cells in the G0/G1 phase, whereas increased the number of cells in S phase.

cells, and the transfection efficiency was detected. We found that all three lines of si-lncRNA 00152 showed high transfection efficiency, with si-lncRNA 00152-1 the most significant (Figure 2B). Meanwhile, HB611 cells were transfected with pcDNA3.1 vector or pcDNA3.1-lncRNA 00152, respectively. The expression of lncRNA 00152 in HB611 cells transfected with the pcDNA3.1-lncRNA 00152 was remarkably higher than those transfected with the pcDNA3.1 vector (Figure 2C). Furthermore, we determined the regulatory effect of lncRNA 00152 on cell proliferation by EdU assay. Results indicated that lncRNA 00152 overexpression remarkably stimulated the proliferative ability of HB611 cells. However, the lncRNA 00152 knockdown in MHCC97 cells markedly inhibited the proliferative ability (Figure 2D). Moreover, we also evaluated the role of lncRNA 00152 in the cell cycle of HCC. Knockdown of lncRNA 00152 in MHCC97 cells significantly increased the number of cells in the G0/G1 phase, whereas significantly decreased the number of cells in S phase. This proved that cell cycle was arrested (Figure 2E). Furthermore, overexpression of lncRNA 00152 in HB611 cells

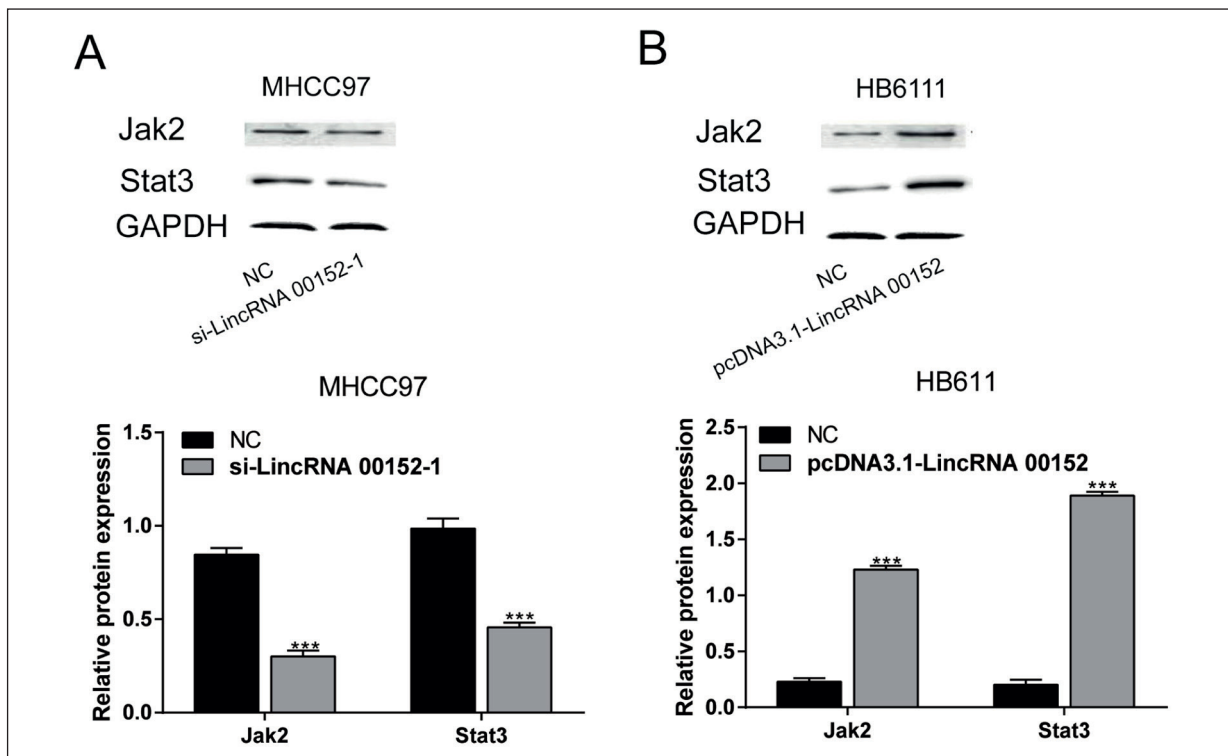
significantly stimulated cell cycle, manifesting as less cells in the G0/G1 phase and more cells in S phase (Figure 2F).

### ***LncRNA 00152 Activated the JAK/STATS Pathway in HCC***

To verify the specific role of the JAK/STATS pathway in the occurrence and progression of HCC, we conducted Western blot to access the relationship between the JAK/STATS pathway and lncRNA 00152. Results showed that, after lncRNA 00152 knockdown in MHCC97 cells, the protein expression levels of JAK2 and STAT3 were remarkably decreased (Figure 3A). However, lncRNA 00152 overexpression in HB611 cells significantly upregulated the protein expression levels of JAK2 and STAT3 (Figure 3B). The above results all elucidated that high expression of lncRNA 00152 promoted HCC development by activating the JAK2/STAT3 pathway.

### **Discussion**

LncRNA was originally considered as “transcript noise”. In a report, they have been found



**Figure 3.** LncRNA 00152 activated the JAK/STATS pathway in HCC. **A**, After lncRNA 00152 knockdown in MHCC97 cells, the protein expression levels of JAK2 and STAT3 were remarkably decreased. **B**, LncRNA 00152 overexpression in HB611 cells significantly upregulated the protein expression levels of JAK2 and STAT3.

to be involved in regulating gene expression and disease development<sup>21</sup>. According to the location of lncRNA in the genome, it can be divided into three categories, namely large intergenic non-coding RNA (lncRNA), antisense lncRNA, and intronic transcript<sup>22</sup>. Meanwhile, lncRNA shows better interference effect than other types of lncRNAs, which is then selected in this study<sup>23</sup>.

Accumulating evidence has shown that lncRNA 00152 is closely related to cell growth, apoptosis, and tumorigenesis. Some studies have also found that the expression of lncRNA 00152 is significantly increased in gastric cancer tissues and cell lines. lncRNA 00152 expression has been demonstrated to be positively correlated with the proliferation and invasion of gastric cancer cells<sup>24</sup>. lncRNA 00152 downregulation can inhibit the proliferation and colony formation, as well as promote cell cycle arrest and apoptosis of gastric cancer cells<sup>25</sup>. Meanwhile, lncRNA 00152 expression is significantly upregulated in tongue squamous cell carcinoma tissues, and is positively correlated with TNM stage of affected patients. High expression of lncRNA 00152 promotes the development of tongue squamous cell carcinoma<sup>26</sup>. Moreover, the expression of lncRNA 00152 is upregulated in gallbladder carcinoma and cell lines, which is negatively correlated with the overall survival of these patients<sup>27</sup>. In the present work, we found that lncRNA 00152 was highly expressed in HCC tissues and cell lines. lncRNA 00152 expression was positively correlated with tumor stage, tumor size, and the overall survival of HCC patients, indicating its diagnostic value for HCC.

lncRNA participates in the regulation of tumor cell proliferation through diverse mechanisms. For example, lncRNA inhibits apoptosis and induces cell cycle arrest, thereby promoting the proliferative ability of tumor cells<sup>28,29</sup>. Meanwhile, lncRNA can also regulate the proliferative ability of tumor cells by mediating the promoter region of tumor-associated genes<sup>30,31</sup>. Immunity of tumor cells can be regulated by lncRNA to achieve the purpose of mediating tumor cell proliferation<sup>32,33</sup>. Furthermore, the proliferative ability of tumor cells can be regulated by lncRNA-induced metabolism changes<sup>34,35</sup>. In the present research, our findings demonstrated that the proportion of HCC cells in G0/G1 phase was significantly decreased after overexpression of lncRNA 00152. This indicated that lncRNA 00152 stimulated the proliferative ability of HCC cells by promoting cell transformation from G0 to S phase.

Dysfunction of proliferation and apoptosis is the major cause for HCC. Current researches have suggested that the JAK/STAT pathway can regulate cell proliferation, apoptosis, and angiogenesis. It has also been confirmed as an important pathway in the pathogenesis of malignant tumors. Particularly, the JAK2/STAT3 pathway is an essential pathway for physiological and pathological reactions in the human body, and is closely related to the occurrence and progression of various tumors. Studies have shown that IL-7 promotes the development of liver cancer by inducing the activation of AKT-dependent IL-6/JAK2/STAT3 pathway<sup>36</sup>. Moreover, activation of the JAK2/STAT3 pathway upregulates the expression level of VEGF and bFGF in non-small cell lung cancer, thereby mediating tumor angiogenesis<sup>36</sup>. Epithelial growth factors induce the migration of ovarian cancer cells by activating the JAK2/STAT3 pathway<sup>37</sup>. Our results showed that highly expressed lncRNA 00152 promoted HCC development by activating the JAK2/STAT3 pathway.

We found that lncRNA 00152 was highly expressed in HCC tissues and cell lines. Overexpression of lncRNA 00152 promoted the proliferation and cell cycle of HCC cells. Western blot results indicated that the JAK2/STAT3 pathway was activated after lncRNA 00152 overexpression, manifesting as upregulated protein expression levels of JAK2 and STAT3.

## Conclusions

We stated that the high expression of lncRNA 00152 promotes the development of HCC by activating the JAK2/STAT3 pathway. Therefore, lncRNA 00152 may serve as a potential hallmark for the diagnosis and treatment of HCC.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

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